Plasma Theophylline Concentrations Measured by High-Pressure Liquid Chromatography

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We present a specific, sensitive high-pressure liquid-chromatographic assay for theophylline in plasma. Only 0.5 ml of plasma is required for each determination, and the lower limit of detection by this method is 0.1 mg/liter. Other xanthines and their metabolites do not interfere. This method is suitable for use in studying the pharmacokinetics of this drug in infants and children, from whom only small volumes of blood are available.

Additional Keyphrase: pediatric chemistry

The original spectrophotometric method for theophylline (1) requires a large (5.0 ml) volume of blood or plasma for each analysis, and appreciable error is involved in determining concentrations less than 5 mg/liter. Also, theophylline is not distinguished from its metabolites or from other xanthines. This is important because metabolites of theophylline are co-extracted in the original method (2). There are gas-liquid chromatographic assays for theophylline (2–4) that require a 1.0 ml plasma sample and are sensitive to 1 mg/liter. High-pressure liquid-chromatographic assays have recently been reported for this drug (5, 6). As little as 2 mg/liter can be detected by ion-exchange (5), but 30 min is required per assay and only a few samples could be analyzed each day. The other liquid-chromatographic method (6) involves reverse-phase chromatography for the separation of theophylline and measures 2.5 to 25 mg/liter.

We have developed a specific assay for theophylline in only a small volume of plasma, a high-pressure liquid-chromatographic method, for which only 0.5 ml of plasma is required for each analysis and which is specific in that the patient need not be withdrawn from other medication or xanthine-containing beverages before sampling. The lower limit of detection by this procedure is 0.1 mg/liter.

Materials and Methods

Reagents

Theophylline was obtained from British Drug Houses (Canada) Ltd., Toronto, Ontario. 3-Methylxanthine and 1,3-dimethyluric acid were obtained from Adams Chemical Co., Round Lake, Ill. 60073. All other chemicals were analytical-reagent grade.

Procedures

Theophylline assay. Pipette 0.5 ml of plasma sample into a 12-ml test tube containing 200 mg of ammonium sulfate. Stir the contents with a vortex-type mixer. Pipette 10 ml of isopropanol/chloroform (5/95 by vol) into the tube. Cap the tube and shake it for 20 min (we used an Eberbach Shaker; Eberbach Corp., Ann Arbor, Mich. 48106). Centrifugse the tube (10 min, 1000 × g). Remove 8.0 ml of the organic (bottom) layer and evaporate with warming in a nitrogen atmosphere (we used an SC/48 Sample Concentrator; Brinkman Instruments Canada Ltd., Toronto, Ontario). Dissolve the residue in 0.1 ml of isopropanol/chloroform (15/85 by vol) and analyze an aliquot of this solution by high-pressure liquid chromatography.

Instrumental conditions. The instrument we used was a Model 4100 high-pressure liquid chromatograph with a variable-wavelength detector (all from Varian Aerograph, Georgetown, Ontario). The detector was set at 273 nm. The 50 cm × 3 mm (i.d.) column was filled with silica gel (10 μm av. particle diameter, Micropak Si 10; Varian Aerograph). The eluting solvent was chloroform/isopropanol/acetic acid (84/15/1 by vol). Solvent flow rate was 40 ml/h. Operating pressure was about 3.45 × 10^6 P.

Preparation of calibration curve. Pipette a standard solution of theophylline in ethanol (5 g/liter) into a known volume of plasma to give a final concentration of 20 mg/liter. Dilute this mixture with plasma to prepare other standards containing 0.5 to 20 mg of theophylline per liter. Extract and analyze these standards in duplicate as described above.
Comparison spectrophotometric method. Spectrophotometric determinations of theophylline in plasma samples were performed according to the method of Schack and Waxler (1).

Statistics. The calibration-curve data were analyzed by a least-squares regression method. This regression analysis was then subjected to analysis of variance, and 95% confidence limits were determined for the data (7).

Two volunteers received an intravenous dose of 5.6 mg of aminophylline per kilogram of body weight, administered during 6 min. We measured disappearance of this drug from the plasma by analysis according to Schack and Waxler (1) and to the present method. We determined the apparent plasma half-life of theophylline by the method of least squares from the slope of the terminal portion of a plot of log concentration vs. time. Blood samples were taken just before the administration of the drug and assayed for the presence of theophylline, to control for any background absorbance that might have been present.

Results and Discussion

Figure 1 illustrates typical chromatograms for normal plasma and for plasma containing theophylline.

Figure 2 shows a representative calibration curve for the analysis of plasma extracts for theophylline. Although this curve represents calibration to only 20 mg/liter, we find that linearity extends to at least 40 mg of theophylline per liter. From the chromatograms, quantitative data are obtained by peak-height analysis. As little as 0.1 mg of this drug per liter in a 0.5-ml sample of plasma can be measured, since multiple samples of plasma that did not contain theophylline showed a recorder deflection equivalent to or less than 50 μg/liter. The lower limit of detection was defined as the minimum quantity of drug in a 20-μl injection that would give a deflection of one unit on the recorder in the area where theophylline eluted (0.05 A full scale; minimum absorbance 0.0005 A). The extraction efficiency for this method was determined to be 78%. Over a six-month period, the coefficient of variation for this analysis was determined to be 2.88% at 5 mg/liter (n = 26) and 3.18% at 10 mg/liter (n = 21). Other ingested xanthines and their metabolites—i.e. caffeine, theobromine, 1,3-dimethyluracil acid, and 3-methylxanthine—do not interfere. As yet we have encountered no drug interference with the assay. This exclusion from interference includes catecholamines, steroids, barbiturates, benzodiazepines, and erythromycin. In Figures 3 and 4,
data are compared for analysis of theophylline concentrations in plasma of the two subjects described by our method, and by the method of Schack and Waxler (1). Figure 3 is the typical semilogarithmic curve for concentration in plasma vs. time curve for the volunteer who does not ingest xanthine-containing beverages. The post-distribution half-life in plasma was not significantly different by the two methods: 9.0 vs. 9.7 h (analysis of variance). However, in a volunteer who ingested xanthine-containing beverages, the data were significantly different by the two methods. This volunteer had not ingested such beverages for 12 h before the investigation and did not ingest coffee or tea for the first 12 h of the study. The post-distribution half-lives differed significantly by the two methods: 11.0 h by our method and 15.7 h by the method of Schack and Waxler (analysis of variance), a difference that would be very important if plasma concentrations were being determined as a basis for selecting an appropriate dosage regimen. This difference cannot be explained by differences in initial background absorbance between the two methods, because blank values were subtracted from drug determinations by the Schack and Waxler method and we have demonstrated no interfering absorbance by our method in the area of the elution curve where theophylline elutes (see Figure 1). In clinical situations, one usually cannot withdraw a patient from other therapy in order to make more accurate determinations of theophylline by the spectrophotometric method.

The new method we have presented offers the following advantages: a smaller volume of plasma is needed for analysis, a smaller volume of extracting solvent, greater sensitivity, and good specificity in the presence of other drugs and xanthines. This method will enable investigation of the kinetic disposition of theophylline to plasma concentrations lower than previously possible and also will allow multiple sampling in infants and children.

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References